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Flavonoids and Spores of *Platyzoma microphyllum*, an Endemic Fern of Australia

ECKHARD WOLLENWEBER and CORNELIA SCHEELE
Institut für Botanik der TH, D-6100 Darmstadt, West Germany
ALICE F. TRYON
Gray Herbarium, Harvard University, Cambridge, MA 02138

This is the first report of flavonoid aglycones in the Australian endemic fern, *Platyzoma microphyllum* R. Br. These are deposited on the leaves as filamentous crystals from glandular trichomes (Barthlott & Wollenweber, 1981). Such lipophilic deposits have been identified on leaves of several genera of the Pteridaceae. They occur in almost all species of *Pityrogramma*, many in *Notholaena*, some in *Cheilanthes*, and a few in *Pellaea* and *Pterozonium* (Wollenweber, 1978). They have been designated as "wax" or "ceraceous indument" in taxonomic literature and as "farinose exudate" or merely as "farina" in phytochemical literature. The term "wax" is incorrect as this material is not a true wax in chemical terms. Extensive studies of the chemical composition of such exudates have revealed that they are usually a mixture of flavonoid aglycones (Wollenweber, 1978), sometimes with considerable amounts of terpenoids (Wollenweber et al., 1982; Arriaga-Giner & Wollenweber, 1986). As part of our continuing studies of the flavonoid excretions (Wollenweber, 1985) we have made a detailed analysis of *Platyzoma microphyllum*. The species is remarkable for the incipient heterosporous condition (A. Tryon, 1964; A. Tryon & Vida, 1967; Duckett & Pang, 1984) and production of filamentous male gametophytes as well as largely archegoniate spatulate gametophytes.

MATERIAL AND METHODS

Specimens were collected at Nicotine Creek, southwest of Mareeba, Queensland, Australia (*R. & A. Tryon 7342*, GH; Wollenweber Herbarium, Darmstadt). The plants were growing in dry *Eucalyptus* scrub, an unusual habitat for ferns (Fig. 1). There were many large clumps growing among sparse grass, on sand that was damp below the surface. A xeric habitat is often characteristic of other species of the Pteridaceae with flavonoid aglycones.

Leaves were washed with acetone to dissolve the lipophilic exudate and the concentrated solution was chromatographed over polyamide. Two flavonoids crystallized from the relevant fractions as fine yellow needles. They were identified by their UV and mass spectra, and these identifications were confirmed by direct comparisons with authentic samples. Six minor flavonoids were identified by co-chromatography with markers (for details of experimental routine procedures see, e.g., Wollenweber et al., 1985).

Results

The spores are of two sizes, with the larger ones 162–190 μm and usually 16 per sporangium. The small spores are 65–105 μm and usually 32 per sporangium. The size differences are apparent in the sample illustrated (Fig. 3). A granulate deposit often covers the reticulate surface in both spore types. There are a series of parallel ridges in the equatorial region of the large spores (Fig. 4) that are absent in the small ones. The development of morphologically distinct gametophytes from the two spore types and origin of heterospory were examined by Duckett and Pang (1984).

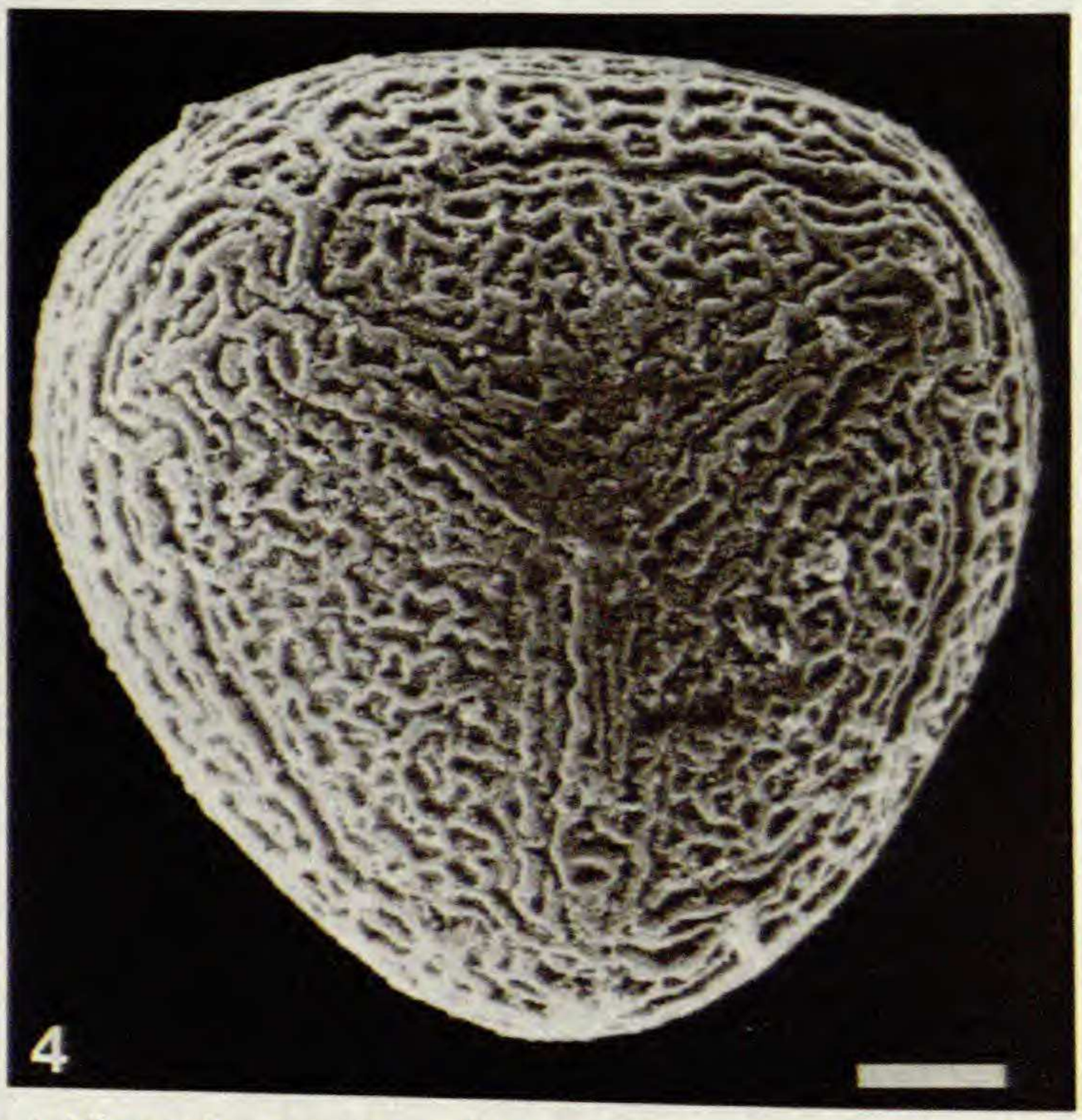
The leaves have indeterminate growth with two rows of coriaceous, pouch-like pinnae adjacent to the rachis (Fig. 2). Two- or three-celled, capitate glands are abundant on the pinnae, which usually have accumulations of yellowish exudate on the surface. Freshly collected specimens have a characteristic scent and, when pressed, leave an oily stain on paper. The odor and stain might be due to the presence of essential oils of some kind. The flavonoid aglycones that cause the yellow color of the leaf exudate were analyzed.

Two major flavonoids were obtained in crystalline form. They were found to be 2′,6′-dihydroxy-4′-methoxy chalcone (Fig. 5a) and 2′,6′-dihydroxy-4′,5′-dimethoxy chalcone (pashanone, Fig. 5b). As minor components, we identified the flavanone pinocembrin-7-methyl ether (pinostrobin, Fig. 5c) and the flavanols galangin-7-methyl ether (izalpinin, Fig. 5d), galangin-3,7-dimethyl ether (Fig. 5e), kaempferol-7-methyl ether (rhamnocitrin, Fig. 5f), kaempferol-3,7-dimethyl ether (kumatakenin, Fig. 5g), and kaempferol-3,7,4′-trimethyl ether (Fig. 5h). Some further trace constituents could not be identified, due to lack of material.

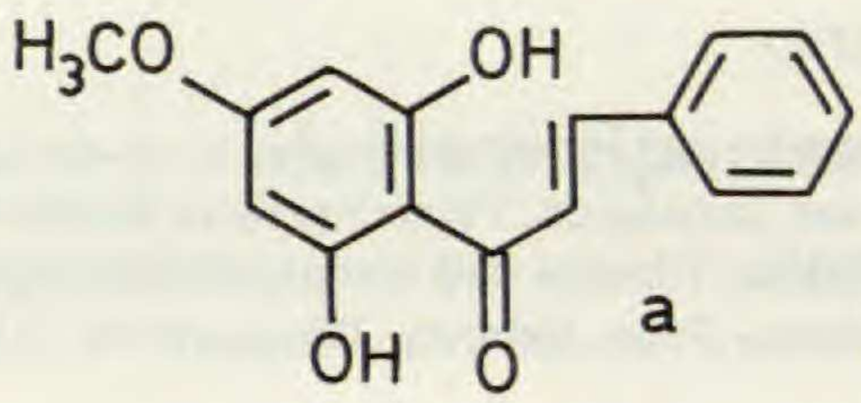
The 2′,6′-dihydroxy-4′-methoxy chalcone is a typical component of yellow or orange leaf exudates in *Pityrogramma* species (except *P. triangularis*; Wollenweber & Dietz, 1980). Several species of *Cheilanthes* and *Notholaena* also produce this chalcone (Wollenweber, 1982a). The rare 2′,6′-dihydroxy-4′,5′-dimethoxy chalcone, pashanone, occurs jointly with the former chalcone as major constituents of the leaf exudate of *Onychium siliculosum* (Desv.) C. Chr. (Wollenweber, 1982b). Pinocembrin-7-methyl ether is assumed to be an artifact, derived from the first mentioned chalcone by cyclization. The methylated flavonols (Figs. 5d–h) are common constituents of the ceraceous indument in *Cheilanthes* and *Notholaena* species and in some varieties of *Pityrogramma triangularis* (Smith, 1980). They were also encountered, in trace amounts, in the thin epicuticular layer on glaucous leaflets of several *Pellaea* species (Wollenweber, 1982a).

Discussion

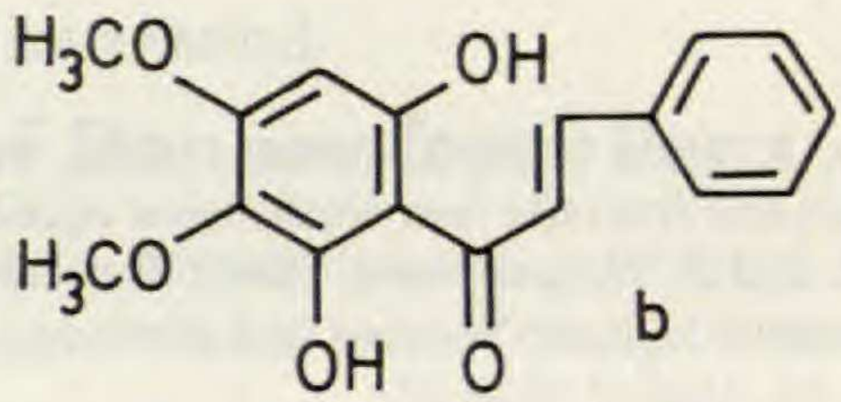
The systematic position of this fern has long been in question, due to its unusual leaf morphology, incipient heterosporous condition, and two forms of gametophyte. It was initially placed in the Gleicheniaceae by Robert Brown in 1810 or considered a separate family, Platyzomataceae, in the Gleicheniales (Nakai, 1950). It was treated as subfamily *Platyzomatoideae* in the Polypodiaceae



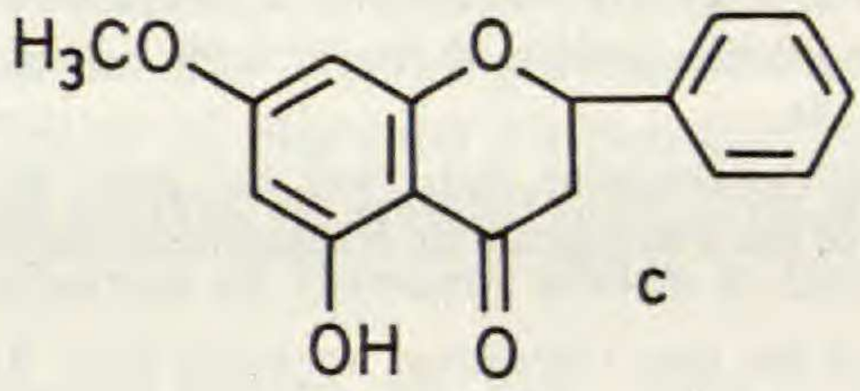
FIGS. 1–4. *Platyzoma microphyllum*. FIG. 1. Three clumps of the plants with *Eucalyptus* trees in the background, near Mareeba, Queensland, Australia. FIG. 2. Part of filiform leaf with pouch-like pinnae, bar = 2 mm. FIG. 3. Small spores and three large ones below, bar = 500 μm. FIG. 4. Proximal face of large spore, parallel ridges in the equatorial region. Queensland, Australia, *Brass & White 64* (GH), bar = 25 μm.



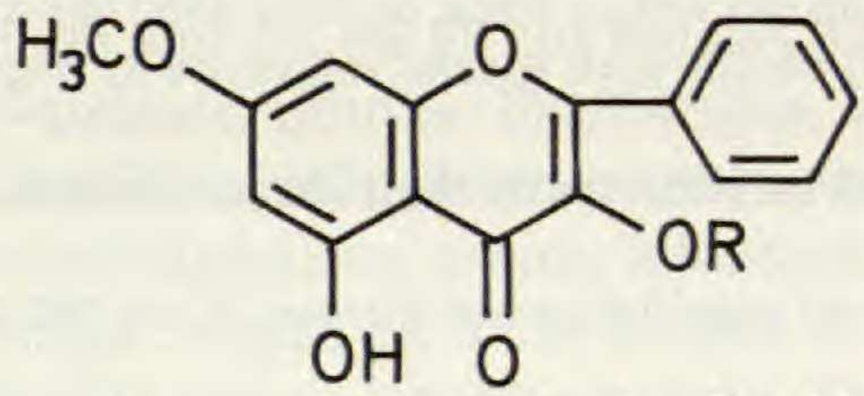
a



b

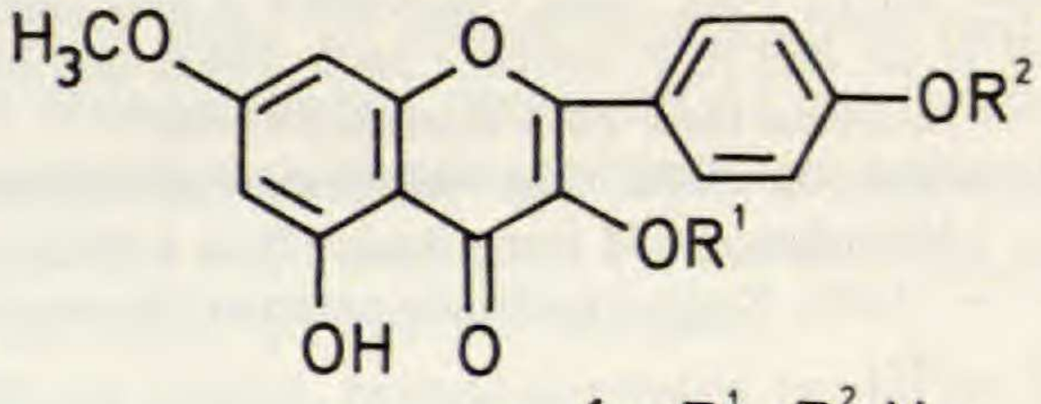


c



d : R = H

e : R = CH_3



f : $R^1 = R^2 = H$

g : $R^1 = CH_3$, $R^2 = H$

h : $R^1 = R^2 = CH_3$

FIG. 5. Flavonoid aglycones that cause the yellow color of the leaf exudate in *Platyzoma microphyllum*: a—2′,6′-dihydroxy-4′-methoxy chalcone, b—pashanone, c—pinostrobin, d—izalpinin, e—galangin-3,7-dimethyl ether, f—rhamnocitrin, g—kumatakenin, h—kaempferol-3,7,4′-trimethyl ether.

(A. Tryon 1961, 1964), and in the tribe *Platyzomateae* in the Pteridaceae (R. & A. Tryon, 1982). Flavonoid aglycones that are excreted and accumulated on the leaf surface have so far been reported for representatives of the genera *Pityrogramma* and *Pterozonium* (tribe Taenitideae), for *Cheilanthes*, *Notholaena*, *Pellaea*, *Negripteris*, *Sinopteris*, and *Onychium* (tribe Cheilantheae), and for *Adiantum* (tribe Adianteae) (Wollenweber, 1979).

Several characteristics of the plants as the medulated protostele, sporangium morphology, and especially the chromosome report of $n = 38$ suggest connections with the Schizaeaceae (A. Tryon & Vida, 1967). The incipient heterosporous condition, known in several unrelated families as the Selaginellaceae, Marsileaceae, and Salviniaceae, has undoubtedly developed independently in *Platyzoma*. The presence of flavonoid aglycones, such as those forming the lipophilic leaf exudate in *Platyzoma microphyllum*, are thus far known only in the Pteridaceae. This supports its treatment in that family, while the unusual morphology, chromosome number, and heterosporous condition support recognition of the species in a separate tribe.

ACKNOWLEDGMENTS

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